REMARKS

I. Amendment of the Claims

Clam 43 has been amended to specify that A consists of "between 2 and 8 locked nucleotide units". Support for this amendment is found on page 8 of the PCT application where it states that "A has a length of 2-10 (preferably 2-8...) ... nucleotide units". Support is also found on page 7 of the PCT application where it is explained that A can include locked nucleotides of various types: "A represents a sequence comprising at least two consecutively located locked nucleotide units, at least one of which is an alpha-L-oxy-LNA unit, and which sequence optionally contains one or more ... non-locked nucleotide units .... and/or optionally contains one or more (such as 2, 3, 4, or 5) locked nucleotide units, such as a unit selected from the group consisting of oxy-LNA, thio-LNA, amino-LNA (all in with alpha-L or beta-D configuration) and derivatives thereof. (emphasis added)

Claim 43 has been amended to specify that B consists of no more than 10 nucleotide units. Support for this amendment is found on page 8 of the PCT application where it states that "B has a length of 1-10 ... nucleotide units".

Claim 43 has also been amended to specify that "B comprises only 3, 4, or 5 contiguous 2'-deoxy-erythro-pentofuranosyl nucleotide units" Support for this amendment is found, for example, on page 8 of the PCT application where it is explained that B can have a subsequence of "at least three nucleotide units having 2'-deoxy-erythro-pentofuranosyl sugar moieties, such as 4, 5, 6, 7, 8, 9, or 10 nucleotide units."

Clam 43 has been amended to specify that C consists of "between 2 and 8 locked nucleotide units". Support for this amendment is found on page 8 of the PCT application where it states that "C ... has a length of 2-10 (preferably 2-8...) ... nucleotide units." Support is also found on page 7 of the PCT application where it is explained that C can include locked nucleotides of

various types: "C represents a sequence comprising at least two consecutively located locked nucleotide units, at least one of which is an alpha-L-oxy-LNA unit, and which sequence optionally contains one or more ... non-locked nucleotide units .... and/or optionally contains one or more (such as 2, 3, 4, or 5) locked nucleotide units, such as a unit selected from the group consisting of oxy-LNA, thio-LNA, amino-LNA (all in with alpha-L or beta-D configuration) and derivatives thereof. (emphasis added)

An example of a molecule within presently amended claim 43 is 2023-t depicted in Table 8 (page 38 of the specification). This molecule consists of, on order: (i) 4 LNA (region A); (ii) 8 nucleotides (region B) that consist of 4 DNA followed by an alpha-oxy-LNA and then 3 DNA; and (c) 5 LNA (region C).

II. Rejections under 35 U.S.C. §112, second paragraph

Claims 69, 70 and 72 were rejected as indefinite for depending from a cancelled claim. Claims 69 and 70 have been amended to correct their dependency. Claim 72 depends from claim 70. In view of the forgoing, Applicant respectfully requests that this rejection be reconsidered and withdrawn.

III. Rejections under 35 U.S.C. §112, first paragraph (written description)

Claims 43-47, 54-60, 62, 65-67 and 69-72 were rejected as failing to meet the written description requirement for including new matter. The Examiner stated that the specification did not support amending claim 43 to: 1) specify that A and C consist of between 2 and 5 nucleotide units; 2) specify that B consist of between 5 and 10 nucleotide units; and 3) specify that one or both of A and C comprise at least two consecutive locked nucleotide units.

Claim 43 has been amended to remove the language referred to by the Examiner. In view of the forgoing, Applicant respectfully requests that this rejection be reconsidered and withdrawn.

IV. Obviousness-type double patenting

Claims 43-47, 54-60, 62, 65-67 and 71 were rejected for obviousness-type double patenting as unpatentable over claim 1-26 of U.S. Patent No. 7,687,617. Applicant will address this rejection upon notification that the claims in the present application are otherwise allowable.

V. Rejections under 35 U.S.C. §103

The Examiner rejected claims 43-47, 54-60, 62, 65-67 and 71 as obvious in view of Kurreck et al. taken with Keinicke et al., Sorensen et al., Orum et al., Wahlestedt et al., and Monia et al.

According to the Examiner, Kurreck teaches LNA/DNA mixmers, gapmers and endblocks; Keinicke teaches that alpha-L-oxy LNA have improved hybridization properties compared to standard DNA oligonucleotides; Sorensen teaches that "α-L-oxy-LNA-containing oligonucleotides are capable of triggering RNase H mediated degradation of a complementary RNA target" and recommend their use as antisense oligonucleotides; and Orum teaches methods for making and using alpha-L-oxy LNA-containing antisense oligonucleotides. In addition, the Examiner noted that Wahlestedt teaches that an LNA-DNA-LNA gapmer oligonucleotide targeted to a rat opiod receptor was effective in reducing receptor expression. Based on these teachings the Examiner concluded that the present invention would be obvious to one of ordinary skill in the art. Applicant respectfully traverses this rejection.

It is Applicant's position that one skilled in the art would not have arrived at the presently claimed oligonucleotides because one skilled in the art would have expected that an alpha-L-oxy LNA, like a beta-D-oxy LNA, interrupting the stretch of deoxy-erythro-pentofuranosyl nucleotide units in a gapmer oligonucleotide such that the oligonucleotide has no more than 3, 4, 5 contiguous deoxy-erythro-pentofuranosyl nucleotide units (as required by the present claims) would sharply diminish the effectiveness of the oligonucleotide as an antisense molecule.

As shown in the present application, Applicant went against this expectation and designed gapmer oligonucleotides in which alpha-L-oxy LNA were included in the DNA stretch of a gapmer oligonucleotide such that there were as few as 5 or 3 contiguous deoxy-erythro-

pentofuranosyl nucleotide units. Applicant surprisingly found that such molecules can be very effective in reducing expression of a target gene is a cell culture system. These results would not have been expected in view of the teachings of the cited references.

One would not include alpha-L-oxy LNA in the gap of a gapmer because LNA decrease RNaseH mediated cleavage when included in the gap

Kurreck conducted an analysis of the effect of gap size on RNaseH-mediated cleavage and found when the contiguous stretch of DNA in a gapmer was less than 8 nucleotides, RNaseH-mediated cleavage decreased. Kurreck concluded that "a DNA gap in a chimeric LNA/DNA oligonucleotide is needed to recruit RNase H." (page 1913, right column) Moreover, Kurreck concluded that "a stretch of 7-8 nt in LNA gapmers is needed for full activation of RNase H" (page 1913, right column). Thus, one skilled in the art would be deterred from including LNA in the gap region of a gapmer because RNAaseH activity, which is very important for the effectiveness of an antisense oligonucleotide, would be diminished. Moreover, to the extent that one would view alpha-L-oxy LNA to be similar to beta-D-oxy LNA, one would be likewise deterred from including alpha-L-oxy LNA in the gap region of a gapmer. Given the teachings of Kurreck, the primary reference cited by the Examiner, one certainly would not have been motivated to generate a gapmer molecule with only 3, 4 or 5 contiguous DNA, a required by the present claims.

The view that inclusion of alpha-L-oxy LNA in oligonucleotides is not compatible with efficient RNaseH cleavage is reinforced by **Sorensen**, who can also be seen as teaching that alpha-L-oxy LNA are not compatible with efficient RNaseH-mediated cleavage. Sorensen reported that an alpha-L-oxy LNA/DNA could elicit RNaseH-mediated cleavage. However, the cleavage was "very slow" and required high enzyme concentrations (page 2164). It should be noted that Sorensen's limited ability to elicit RNaseH-mediated cleavage using an alpha-L-oxy LNA/DNA molecule was achieved *in vitro*, where it was possible to manipulate the level of RNaseH.

It is true that **Wahlestedt** reports that a LNA/DNA mixmer exhibited some limited ability to recruit RNaseH, but this result shows that the mixmer is much less effective than the DNA/LNA gapmer. This can be seen in Figure 4, which shows a DNA oligonucleotide and a LNA/DNA gapmer cleave the RNA target *in vitro* within 10 minutes. However, the LNA/DNA mixmer elicited almost no cleavage even after 60 minutes. Moreover, even the limited RNaseH cleavage found by Wahlestedt could not be later confirmed. Kurreck, discussed above, stated that they could not confirm the results of Wahlestedt that LNA/DNA mixmers could elicit some RNaseH mediated cleavage (page 1914, first full paragraph). Indeed, Kurreck stated that for the LNA/DNA mixmer, there was no significant RNaseH cleavage even after 60 minutes (page 1914, right column).

Neither **Orum** nor **Monia** teach that a gapmer that includes alpha-L-oxy LNA such that the gap of the oligonucleotide has no more than 3, 4, 5 contiguous deoxy-erythro-pentofuranosyl nucleotide units would be effective. The Examiner has not suggested otherwise.

The cited references, taken as a whole, would deter one from including an alpha-L-oxy LNA in the DNA gap of a gapmer oligonucleotide because doing so would sharply diminish the RNaseH-mediated cleavage that is vital for the *in vivo* effectiveness of a gapmer antisense oligonucleotide.

One would not substitute alpha-L-oxy LNA for beta-D-oxy LNA in the oligonucleotides of the cited references because, according to Keinicke, alpha-L-oxy LNA have reduced affinity for RNA compared to beta-D-oxy LNA

While it is true that certain of the cited references teaching alpha-L-oxy LNA suggest that such nucleotides merit consideration for inclusion in antisense oligonucleotides, none teach that they should be placed in the gap of gapmer oligonucleotide. Moreover, certain of the references suggest that alpha-L-oxy LNA are not as advantageous as LNA. Thus, **Keinicke** teaches that incorporation of three alpha-L-oxy LNA into a DNA oligonucleotide increases affinity towards

RNA to lesser extent than incorporation of three beta-D-oxy LNA (Table 1 comparing 2 and 3 to 1 and 7 and 8 to 6).

In fact, **Keinicke** having studied alpha-L-oxy LNA and alpha-L-oxy RNA, suggest that the latter, alpha-L-oxy RNA/alpha-L-oxy LNA chimeras, not alpha-L-oxy LNA/DNA chimeras be studied further for use as antisense molecules (page 595, last paragraph).

Taken as a whole, **Keinicke** would discourage one from replacing a beta-D-oxy LNA with an alpha-L-oxy LNA because one would expect to see a decrease in binding affinity. This is a second reason that one of ordinary skill in the art would not incorporate an alpha-L-oxy LNA into the gap of a gapmer oligonucleotide.

One skilled in the art would not place an alpha-L-oxy LNA in the gap of a gapmer oligonucleotide

The cited prior art, taken as a whole, suggests that one should **not** place an alpha-L-oxy LNA in the gap of a gapmer oligonucleotide because doing should would, like placing an LNA in the gap of a gapmer, reduce RNaseH-mediated cleavage. In addition, the prior art suggests that alpha-L-oxy LNA do not increase affinity towards RNA to the extent that LNA does. For this separate reason, one skilled in the art would not place an alpha-L-oxy LNA in the gap of a gapmer.

Applicant surprisingly found that an alpha-L-oxy LNA placed in the gap of a gapmer does not decrease the effectiveness of the oligonucleotide as an antisense oligonucleotide

Data presented in the present specification demonstrates that, contrary to what would be expected, an alpha-L-oxy-LNA interrupting a run of DNA in an antisense gapmer, does not severely diminish the effectiveness of the antisense gapmer. This can be seen by considering the antisense gapmers in Table 8 of the present application. The third oligonucleotide listed in Table 8 (4-3-1-3-5a; 2023-t) has 4 beta-D-oxy LNA followed by 3 DNA, 1 alpha-L-oxy LNA, 3 DNA

and 5 beta-D-oxy LNA. Thus, this molecule has an uninterrupted stretch of DNA that is only 3 nucleotides long. Based on the behavior of beta-D-oxy-LNA described in the cited references, one would expect that this oligonucleotide, 4-3-1-3-5a, would have no or extremely poor activity. Despite this expectation, this oligonucleotide significantly reduced expression of a luciferase target by 90% (see Figure 16). In fact, the reduction in luciferase expression was similar to a gapmer oligonucleotide of the same sequence in which the DNA gap was not interrupted by an alpha-L-oxy LNA (i.e., a molecule having a gap containing a run of 7 contiguous DNA; see Figure 16, "gapmer all-PS"). This result is surprising in view of the cited prior art references. An antisense oligonucleotide identical to 4-3-1-3-5a except for the fact that the beta-D-oxy LNA on the inside of each flank were replaced with alpha-L-oxy LNA (4-3-1-3-5b; 2023-u; Table 8, fourth entry) was also very effective (see Figure 16) despite the fact that the uninterrupted stretch of DNA is only 3 nucleotides long. Finally, an oligonucleotide with two alpha-L-oxy LNA replacing DNA in a 9 nucleotide DNA gap, leaving a run of only 5 contiguous DNA, was also effective (see Table 8, second entry (4-1-1-5-1-1-3b; and Figure 16).

The results described in the specification are surprising in view of the prior art, and these results were achieved by doing what the prior suggested should not be done – interrupt the DNA gap region of a gapmer with a locked nucleic acid. Despite the reasonable expectation that such molecules should have sharply reduced efficacy, Applicants found that they are effective.

In view of the forgoing, Applicant respectfully requests that the rejections under 35 U.S.C. §103 be reconsidered and withdrawn.

CONCLUSION

It is believed that the claims are in condition for allowance.

The fees in the amount of $1110.00 for the Petition for Extension of Time are being paid concurrently herewith on the Electronic Filing System (EFS) by way of Deposit Account authorization. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 22460-0003US1.

Respectfully submitted,

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